

## REMARKS

Examination of claims 1-14 is reported in the present Office Action. Claims 1-14 were rejected under 35 U.S.C. § 112, second paragraph; claims 8-9 were rejected under 35 U.S.C. § 101; claims 1, 4-10, and 13-14 were rejected under 35 U.S.C. § 102(b); claims 1-14 were rejected under 35 U.S.C. § 103(a); and claims 1-14 were rejected under the judicially-created Doctrine of Obviousness-Type Double Patenting. Applicants' specification was also objected to for failing to include updated priority information and select sequence identifiers. Claims 8 and 9 were objected to as lacking antecedent basis. These rejections and objections are addressed as follows.

### Priority

Applicants have amended the specification to update the status of the parent application and to indicate that the present application is a divisional of U.S. Serial No. 09/371,338 (now U.S. Patent No. 6,613,959 B1).

### Sequence Listing

The Office has objected to Applicants' specification under 37 C.F.R. § 1.821(d) as failing to provide a sequence identifier for individual sequences identified in Fig. 11. To address this issue, Applicants have amended the specification to include sequence identifiers for sequences found on page 18 (lines 15-20). In addition, applicants submit

an amended sequence listing that includes the amino acid sequences of ANP1L and ANP1S. No new matter has been added.

#### Claim Objections

Applicants have amended claims 8 and 9 to refer to -- the plant -- as suggested by the Office.

#### Rejection Under 35 U.S.C. § 112, second paragraph

Claims 1-14 stand rejected under 35 U.S.C. § 112, second paragraph as being indefinite. In view of the present amendments to claims 1-5 and 10-11, these rejections should be withdrawn.

#### Rejection Under 35 U.S.C. § 101

Claims 8-9 stand rejected under 35 U.S.C. § 101 on the ground that the claimed invention is directed to non-statutory subject matter. In view of the present amendments to claims 8 and 9, these rejections should be withdrawn.

#### Rejection Under 35 U.S.C. § 102(b)

Claims 1, 4-10, and 13-14 were rejected under 35 U.S.C. § 102(b) as anticipated by Tanksley *et al.* (U.S. Patent No. 5,648,599).

Claim 1, as amended, and claims 4-10 and 13-14, which refer directly or indirectly to claim 1, are now drawn to a plant that includes recombinant nucleic acid that encodes a polypeptide comprising a constitutively-active kinase domain of a mitogen-activated protein kinase kinase kinase (MAPKKK) or a kinase domain thereof. Support for this amendment is found in the specification, for example, on page 21, in the section under the heading of “Constitutional Active NPKI Represses Anxin-Inducible Promoters.”

The Tanksley teaching is limited to the *Pto* gene and the *Pto* gene is not a MAPK, a MAPKK, or even a MAPKKK. Furthermore, the *Pto* gene does not encode a polypeptide having a regulatory domain that can be deleted to render the polypeptide constitutively active as found in MAPKKKs. Accordingly, Tanksley cannot anticipate the claims, as amended, and this rejection should be withdrawn.

#### Rejections Under 35 U.S.C. § 103

Claims 1-14 were rejected under 35 U.S.C. § 103(a) as unpatentable over Tanksley *et al.* (U.S. Patent No. 5,648,599) in view of Mizoguchi *et al.* (PNAS Vol 93, pp. 765-769 (1996)). In applying this rejection, the Office asserts:

It would have been obvious to one of ordinary skill in the art at the time the invention was made to transform a plant with a gene encoding a kinase protein for a desired trait as taught by Tanksley *et al.*, and to modify that method by incorporating any other known kinase encoding gene including an MAPKKK encoding gene taught by Mizoguchi. One having ordinary skill in the art would have been motivated to do this, given the availability of MAPKKK encoding genes and their roles in signal transduction pathway under stress conditions as taught by Mizoguchi *et al.* The claims fail to recite specific structural characteristics (such as percent of identity) that would distinguish the claimed transgene from the prior art gene.

Therefore, the claimed invention as whole was clearly a *prima facie* obvious. For the following reasons, this rejection should be withdrawn.

The test of obviousness *vel non* is statutory. It requires that one compare the claim's "subject matter as a whole" with the prior art "to which said subject matter pertains." 35 U.S.C. §103(a). The inquiry is fact-specific. This is so "whether the invention be a process for making or a process of using, or some other process." *In re Kuehl*, 475 F.2d 658, 665, 177 U.S.P.Q. 250, 255 (C.C.P.A. 1973). When the references cited by the Patent Office fail to establish a *prima facie* case of obviousness, the rejection is improper and must be withdrawn. *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988).

Applicants' presently claimed invention stems, at least in part, from the recognition that expression of a gene encoding a constitutively active MAPKKK confers multi-stress tolerance or resistance in plants. Accordingly, applicants' invention is directed to plants that are protected against a variety of stresses. Because neither Tanksley *et al.* nor Mizoguchi *et al.* disclose or suggest applicants' claimed invention, these references, either alone or in combination, cannot produce the invention, nor render it obvious.

Turning first to Tanksley *et al.*, this reference fails to disclose that Pto is a MAPKKK. In addition, Tanksley fails to disclose that the expression of a constitutively active MAPKKK confers multi-stress tolerance or resistance on a plant. Thus, the Office's contention that it would have been obvious to make plants expressing a

MAPKKK to confer tolerance to an environmental stress is incorrect.

In addition, to the extent that the Office relies upon Tanksley to establish that it would have been obvious to express a gene that encodes a constitutively active MAPKKK to confer resistance or tolerance to a variety of stresses, merely because it would be obvious to try such an experiment, the Office is in error. *See Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 231 U.S.P.Q.2d 81 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987). (“Obvious to try” is improper consideration in adjudicating obviousness issue.) What is needed for obviousness is a reasonable expectation of success. *In re O’Farrell*, 853 F.2d 894, 7 U.S.P.Q.2d 1673 (Fed. Cir. 1988). As a matter of law, the § 103 rejection based on Tanksley should be withdrawn.

Similarly, a conclusion that the invention would have been obvious cannot be properly reached when Tanksley *et al.* is considered in view of Mizoguchi *et al.* Mizoguchi, like Tanksley, does not disclose that expression of a constitutively active MAPKKK renders a plant tolerant or resistant to stress. Indeed, Mizoguchi provides no functional information on the biology of stress tolerance in plants resulting from expression of a constitutively active MAPKKK. Indeed, Mizoguchi, in a later publication, *Trends in Biotechnology* 15:15-19, 1997 (copy enclosed), at page 18 (column 2), makes this point clear:

Analyses of transgenic plants that overexpress or repress genes for MAPKs, MAPKKs, and MAPKKKs **will help to elucidate the molecular mechanisms of the stress response and adaptation to environmental changes in higher plants (emphasis added).**

Thus, it is clear that Mizoguchi was only beginning to investigate whether the expression of a MAPKKK gene might be involved in stress tolerance. Accordingly, Mizoguchi *et al.* cannot teach or suggest what they themselves did not know or recognize, and therefore provides no scientific or logical predicate for rendering applicants' claims obvious.

The Tanksley *et al.* and Mizoguchi *et al.* references are unavailing, and, in combination, cannot support the present obviousness rejection.

### Double Patenting

Claims 1-14 were rejected under the judicially-created Doctrine of Obviousness Type Double Patenting over claims 1-9 of U.S. Patent No. 6,613,959, of which the present application was filed as a continuation. Applicants respectfully submit that the invention of the claims pursued in the present application, 1-14, was indicated by the Examiner in the parent case to be distinct from the invention of the claims that were pursued in the parent application, original claims 1-46 (see paper no. 9 in U.S. Serial No. 09/371,338). Thus, consistent with 35 U.S.C. § 121, applicants respectfully request that the present application be designated as a divisional application, which would thus preclude the patent that issued from the parent application from being cited against it. To effect this change, applicants have herein amended the priority claim to indicate that this application is a divisional application, as well as are submitting herewith a petition to correct the filing receipt for this case. In view of this amendment, applicants respectfully

submit that a terminal disclaimer should not be required in this case and that the present rejection can now be withdrawn.

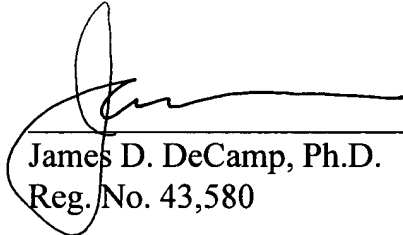
CONCLUSION

Applicant submits that the claims are now in condition for allowance, and such action is respectfully requested.

Enclosed is a petition to extend the period for replying for three months, to and including August 10, 2005. If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 9 August 2005

  
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# Environmental stress response in plants: the role of mitogen-activated protein kinases

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Mitogen-activated protein kinase (MAPK) cascades have essential roles in diverse intracellular signaling processes in plants, animals and yeasts. In plants, transcription of genes encoding protein kinases involved in MAPK cascades is upregulated by environmental stresses and plant hormones; in addition, MAPK-like kinase activities are transiently activated in response to environmental stresses. Consequently, MAPK cascades are now thought to have important roles in stress signal transduction pathways in higher plants.

Mitogen-activated protein kinases (MAPKs) were initially identified as serine/threonine kinases that were activated by various growth factors and in phosphorylation cascade reactions occurring during mitosis in animals<sup>1-3</sup>. MAPKs have key roles in integrating multiple intracellular signals transmitted by various second messengers. MAPK cascades are composed of three protein kinases: MAPKs, MAPK kinases (MAPKKs), and MAPKK kinases (MAPKKKs) (Fig. 1). MAPKs are activated when both tyrosine and threonine residues in the TXY motif are phosphorylated by MAPKKs. MAPKKs are activated when serine and serine/threonine residues in the SxxxS/T motif are phosphorylated by MAPKKKs. MAPK cascades have been shown to function in various signal transduction pathways in animals and yeasts<sup>1-5</sup> (Fig. 2). In plants, many cDNAs for MAPKs, MAPKKs and MAPKKKs have been isolated from various species. A larger number of MAPKs have been identified in plants than in animals and yeasts, which suggests that MAPK cascades have a wider variety of roles in plants. However, their functions are not yet clear. Recently, it has been demonstrated that both MAPK-like kinase activity and mRNA levels of the components of MAPK cascades increase in response to environmental stress in plants. This suggests that some of the plant MAPK cascades have important roles in environmental stress responses and plant hormone signal transduction.

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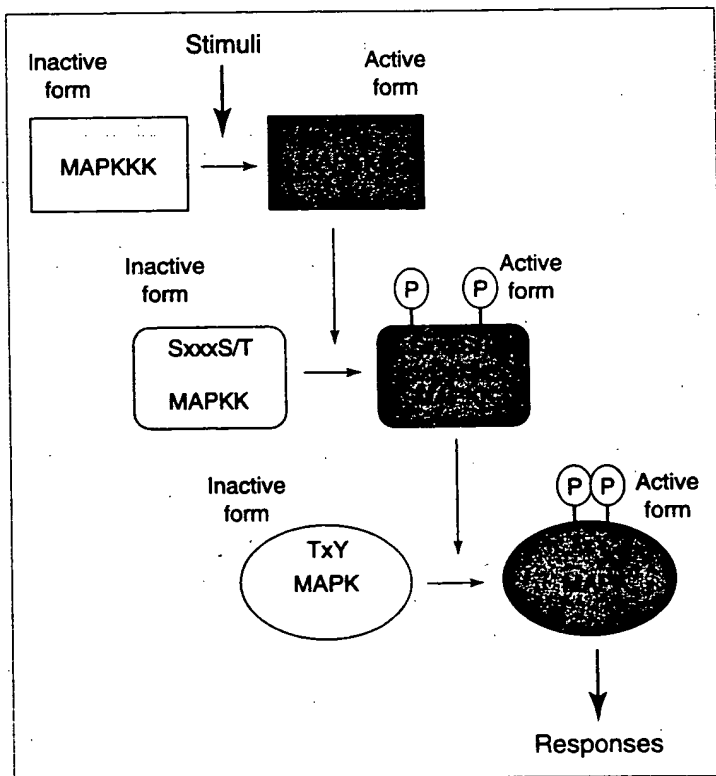


Figure 1

Regulation of the mitogen-activated protein kinase (MAPK) cascade by phosphorylation. An inactive, dephosphorylated MAPK is activated by phosphorylation of both threonine and tyrosine residues in the sequence Txy, which is catalyzed by an immediate upstream MAPK kinase (MAPKK). An inactive, dephosphorylated MAPKK is activated by phosphorylation of both serine and serine/threonine residues in the SxxxS/T sequence, which is catalyzed by an immediate upstream MAPKK kinase (MAPKKK).

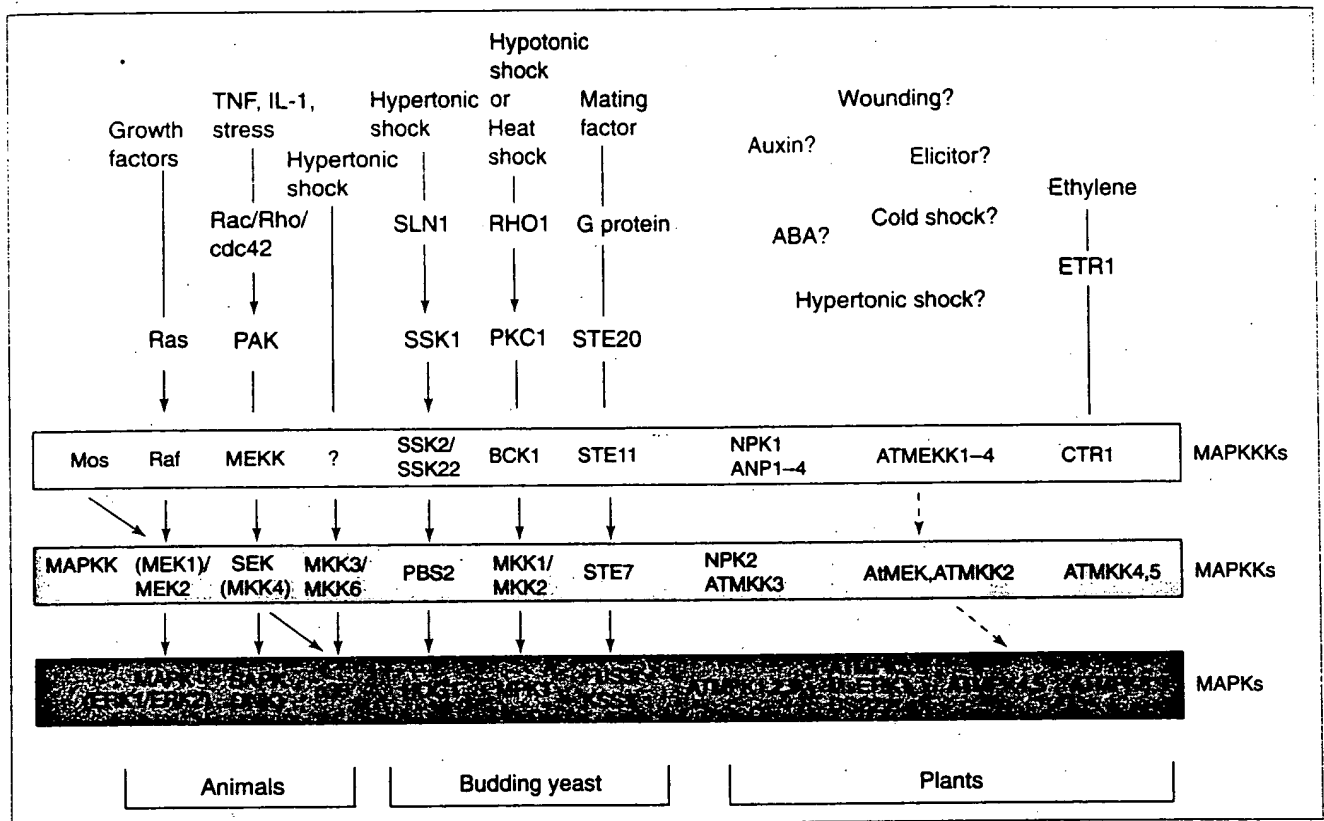


Figure 2

A hypothetical model for mitogen-activated protein kinase (MAPK) cascades in various signal transduction pathways in animals, yeasts and plants. There are at least four MAPK cascades in animals and at least five MAPK cascades in *Saccharomyces cerevisiae*. Numerous MAPKKK/MAPKK/MAPK homologs have been identified in plants. Plant MAPKs are classified into four subgroups. See text for details.

### MAPK cascades and stress responses in animals

In animal cells, at least four different MAPK cascades function in the different signaling pathways (Fig. 2). A cascade involving Raf/Mos, MEK1/MEK2 and ERK1 operates in growth-factor-dependent cell proliferation. The response to stresses such as UV irradiation, translational inhibitors, heat shock and tumor necrosis factor involves a cascade comprising MEKK, SEK/MKK4 and SAPK/JNK, whereas environmental stresses such as osmotic shock and UV irradiation involve the cascade MKK3/MKK6/MKK4 and p38 (Refs 1-3,6). Recently, a new MAPKK homolog, MEK5, was isolated by polymerase chain reaction (PCR), and a new MAPK homolog, ERK5, was identified by a specific interaction with MEK5 in the yeast two-hybrid system<sup>7</sup>. The MEK5-ERK5 pathway is the fourth candidate of the animal MAPK cascades. The animal MAPKs, ERK1, ERK2 and ERK5 all share the conserved phosphorylation site motif TEY (Refs 1-3,7). By contrast, SAPK/JNK and p38 have the motif TPY and TGY, respectively<sup>1-3,6</sup>.

### MAPK cascades and stress responses in yeasts

In *Saccharomyces cerevisiae*, at least five different MAPK signaling pathways have been described<sup>1-3</sup>: (1) the mating-pheromone-response pathway (STE11, STE7 and FUS3/KSS1); (2) the protein kinase C (PKC)-

dependent signaling pathway, which has a role in maintenance of cell wall integrity (BCK1, MKK1/MKK2 and MPK1); (3) the osmoregulatory pathway (SSK2/SSK22, PBS2 and HOG1); (4) the pseudohyphal differentiation pathway (STE11 and STE7); and (5) the spore wall assembly pathway (SMK1). In *Schizosaccharomyces pombe*, at least two different MAPK signaling pathways have been described<sup>1-3,8</sup>: the mating-pheromone-response pathway (byr2, byr1 and spk1); and the osmoregulatory pathway (wis1 and sty1). The yeast MAPKs FUS3, KSS1, MPK1 and spk1 have the motif TEY at the phosphorylation site<sup>1-3</sup>, whereas HOG1 and sty1 have the motif TGY, and SMK1 has the motif TNY (Refs 1-3,8).

When yeast cells are exposed to high osmolarity, glycerol synthesis is induced to increase internal osmolarity and the MAPK cascade involving SSK2/SSK22, PBS2 and HOG1 is activated. Maeda *et al.* have identified new eukaryotic components (SLN1 and SSK1) that function as direct upstream factors of this MAPK cascade<sup>1-3</sup>. In the 'two-component regulatory system' SLN1p and SSK1p have high sequence identity to the sensor and regulator factors, respectively. Two-component regulatory systems are widespread in bacteria and function in various signal transduction pathways. Maeda *et al.* also demonstrated that SHO1p directly interacts with and activates PBS2p in a different

pathway from the Sln1-Ssk1-Ssk2/Ssk22 pathway<sup>9</sup>. Recently, Davenport *et al.* have shown that the BCK1-MKK1/MKK2-MPK1 pathway functions in the hypotonic shock response<sup>10</sup>. These observations indicate that the MAPK cascades in yeasts have important roles not only in mating and developmental signaling processes but also in mediating cellular responses to environmental stresses.

#### Plant homologs of protein kinases in MAPK cascades

A number of genes for MAPKs have been reported in higher plants<sup>4,5,11-21</sup> (Table 1). We have demonstrated in *Arabidopsis thaliana* that MAPKs constitute a gene family with at least nine members (ATMPK1-9), which can be classified into four subgroups based on phylogenetic analysis of their amino acid sequences<sup>11,12</sup> (Mizoguchi and Shinozaki, unpublished). ATMPK8 and ATMPK9 are classified into the fourth new subgroup. Instead of the motif TEY on animal ERKs, ATMPK8 and ATMPK9 contain the sequence TDY (Mizoguchi and Shinozaki, unpublished).

NPK2, which is structurally related to MAPKK, has been isolated from tobacco<sup>5,22</sup>; NPK2-related genes have been revealed in various plant species by Southern blot analysis, and two types of *Arabidopsis* cDNA clones that hybridize with NPK2 have been isolated<sup>5</sup>. Recently, Morris *et al.* have identified one, AtMEK (P. Morris and J. Giraudat, pers. commun.), and we have identified four additional *Arabidopsis* cDNAs, ATMKK2-4 (Ichimura, Mizoguchi and Shinozaki, unpublished), encoding protein kinases that have significant homology to MAPKKs. These plant MAPKK homologs can be classified into three subgroups based on phylogenetic analysis of their amino acid sequences (Ichimura, Mizoguchi and Shinozaki, unpublished).

Three types of MAPKKK homologs have been isolated from plants. The CTR1 gene, encoding a Raf homolog that functions as an activator of MAPKK in animal cells<sup>1-3</sup>, has a key role in the ethylene signal transduction pathway<sup>23</sup>. NPK1 (Ref. 24), which is structurally related to MAPKKKs, has been isolated from tobacco and shown to complement the growth defect of the yeast *bck1Δ* mutant. Nishihama *et al.* have isolated three cDNAs (ANP1-3) from *Arabidopsis* that encode homologs of NPK1 (Ref. 5). We isolated *Arabidopsis* cDNAs (ATMEKK1-4) encoding protein kinases that are structurally related to MAPKKKs (such as Byr2, STE11, BCK1, MEKK and NPK1)<sup>25</sup>. ATMEKK1 can complement the yeast *ste11* mutant in response to the mating pheromone<sup>25</sup>.

#### Interactions of the components of MAPK cascades in higher plants

A number of genes for MAPKs and several genes for MAPKKs and MAPKKKs have been reported in higher plants<sup>4,5,11-25</sup>. However, there have been no reports of direct interactions between MAPK and MAPKK or between MAPKK and MAPKKK from plants, and no evidence of direct phosphorylation and

**Table 1. Summary of the isolated cDNAs that encode MAPK, MAPKK and MAPKKK homologs in plants**

Gene name	Plant species	Group <sup>a</sup>	Refs
<b>MAPKs</b>			
ATMPK1	<i>Arabidopsis thaliana</i>	1	11
ATMPK2	<i>Arabidopsis thaliana</i>	1	11
ATMPK7	<i>Arabidopsis thaliana</i>	1	12
NTF3	<i>Nicotiana tabacum</i>	1	13
PMEK1	<i>Petunia hybrida</i>	1	14
ATMPK3	<i>Arabidopsis thaliana</i>	2	12, 25
ATMPK6	<i>Arabidopsis thaliana</i>	2	12
MsERK1 (MsK7)	<i>Medicago sativa</i>	2	15, 16
D5	<i>Pisum sativum</i>	2	17
Asmap1	<i>Avena sativa</i>	2	18
NTF4	<i>Nicotiana tabacum</i>	2	19
NTF6	<i>Nicotiana tabacum</i>	2	19
MMK1	<i>Medicago varia</i>	2	20
DS22	<i>Nicotiana tabacum</i>	2	21
ATMPK4	<i>Arabidopsis thaliana</i>	3	12
ATMPK5	<i>Arabidopsis thaliana</i>	3	12
MMK2	<i>Medicago varia</i>	3	20
ATMPK8	<i>Arabidopsis thaliana</i>	4	<sup>b</sup>
ATMPK9	<i>Arabidopsis thaliana</i>	4	<sup>b</sup>
<b>MAPKKs</b>			
NPK2	<i>Nicotiana tabacum</i>	1	22
ATMKK3	<i>Arabidopsis thaliana</i>	1	<sup>c</sup>
AtMEK	<i>Arabidopsis thaliana</i>	2	<sup>d</sup>
ATMKK2	<i>Arabidopsis thaliana</i>	2	<sup>c</sup>
ATMKK4	<i>Arabidopsis thaliana</i>	3	<sup>c</sup>
ATMKK5	<i>Arabidopsis thaliana</i>	3	<sup>c</sup>
<b>MAPKKKs</b>			
NPK1	<i>Nicotiana tabacum</i>	1	24
ANP1	<i>Arabidopsis thaliana</i>	1	5
ANP2	<i>Arabidopsis thaliana</i>	1	5
ANP3	<i>Arabidopsis thaliana</i>	1	5
ATMEKK1	<i>Arabidopsis thaliana</i>	2	25
ATMEKK2	<i>Arabidopsis thaliana</i>	2	25
ATMEKK3	<i>Arabidopsis thaliana</i>	2	25
ATMEKK4	<i>Arabidopsis thaliana</i>	2	25
CTR1	<i>Arabidopsis thaliana</i>	3	23

<sup>a</sup>MAPK, MAPKK and MAPKKK homologs in plants can be classified into four, three and three subgroups, respectively, based on our phylogenetic analyses.

<sup>b</sup>Mizoguchi *et al.*, unpublished.

<sup>c</sup>Ichimura *et al.*, unpublished.

<sup>d</sup>P. Morris *et al.*, unpublished.

activation either of MAPK by MAPKK or of MAPKK by MAPKKK from plants. Recently, we demonstrated that ATMPK4 specifically interacts with AtMEK in the yeast two-hybrid system (Mizoguchi, Morris and Shinozaki, unpublished). Furthermore, coexpression of ATMPK4 with AtMEK suppressed the *mpk1Δ* (MAPK<sup>-</sup>) and the *bck1Δ* (MAPKKK<sup>-</sup>) yeast mutations (Mizoguchi, Morris and Shinozaki, unpublished). These results suggest that AtMEK (MAPKK) functions as a direct upstream factor of ATMPK4 (MAPK). By using the yeast two-hybrid system and complementation analysis of yeast mutants we have also found

that ATMEKK1 (MAPKKK) not only interacts with but also activates AtMEK (MAPKK) (Mizoguchi, Morris and Shinozaki, unpublished). These observations suggest that ATMEKK1, AtMEK and ATPMK4 constitute a MAPK cascade in *Arabidopsis* cells.

#### MAPK-like activities in plants

Plants have MAPK-like kinase activities with similar biochemical characteristics to those of known MAPKs of yeast and animal cells. Myelin basic protein (MBP) is a good substrate for MAPKs, such as rat ERK1/ERK2 (Refs 1-3), budding yeast Mpk1 (Refs 1-3), alfalfa MsERK1 (Refs 13,14) and *Arabidopsis* ATPMK1/ATMPK2 (Ref. 11). We demonstrated that a 46 kDa protein kinase that phosphorylates MBP was rapidly and transiently activated by the plant hormone auxin analog 2,4-dichlorophenoxyacetic acid (2,4-D) in auxin-starved tobacco BY-2 cells<sup>11</sup>. Protein kinase activities that phosphorylated recombinant ATPMK2 protein were also increased rapidly by 2,4-D treatment in auxin-starved cells<sup>11</sup>. Knetsch *et al.* reported that the plant hormone abscisic acid (ABA) induced a rapid and transient activation of a MAPK-like activity in barley aleurone protoplasts<sup>26</sup>. Suzuki *et al.* also detected a transient activation of a 47 kDa MBP kinase activity in cultured tobacco cells treated with fungus-derived elicitors<sup>27</sup>. This protein kinase is phosphorylated on tyrosine residues, and activated by upstream protein kinase(s) in cultured tobacco cells<sup>27</sup>. Usami *et al.* demonstrated that a 46 kDa protein kinase that phosphorylated MBP was rapidly and transiently activated in the wounded tissues of tobacco<sup>28</sup>. This MBP kinase required the phosphorylation of both tyrosine and threonine/serine residues for its activity. Furthermore, similar protein kinase activities were detected in various plant species<sup>5</sup>. However, to determine if these kinases are MAPKs, the peptide sequences, or biochemical analysis using specific antibodies against the cloned MAPKs, are required. Recently, Bögre *et al.* and Jonak *et al.* used a specific antibody against the alfalfa MAPK MMK4 to demonstrate that it is activated by a variety of stresses including mechanical stimuli, low temperature and drought<sup>29,30</sup>.

#### Transcriptional control of the putative components of plant MAPK cascades in response to environmental stresses

We have demonstrated that the levels of transcripts for three protein kinases, ATMEKK1 (MAPKKK), ATPMK3 (MAPK) and ATPK19 (ribosomal S6 kinase or RSK homolog), increased markedly and simultaneously when plants were treated with touch stimuli, low temperature and salinity stress<sup>12,25</sup>. These results suggest that some of the MAPK cascades in plants function in transducing signals in the presence of environmental stress and that MAPK cascades are regulated at the transcriptional and post-translational level in plants<sup>25</sup>. Recently, Seo *et al.* used differential hybridization to isolate a tobacco cDNA clone (DS22) for a wound-inducible gene encoding a MAPK homolog (WIPK; Ref. 21). The transcript for WIPK

increased rapidly after mechanical wounding. Analysis of WIPK overexpression in transgenic tobacco suggested that WIPK mediates jasmonic acid (JA)- and salicylic acid (SA)-induced wound signal transduction pathways. In the transgenic tobacco, the endogenous WIPK gene was silenced and the production of wound-induced JA was inhibited, followed by suppressed accumulation of wound-inducible gene transcripts. By contrast, the levels of SA and transcripts of SA-inducible, pathogenesis-related proteins increased abnormally upon wounding. The transcript level and kinase activity of MMK4 increased after cold and drought treatments of alfalfa plants<sup>30</sup>. Three MAPKs from different plant species, ATPMK3, WIPK and MMK4, have a higher sequence identity to one another than to other MAPKs, and the mRNA levels of these MAPKs increased after stress treatments, suggesting that these MAPKs play similar roles in plants under a variety of stress conditions. Wilkinson *et al.* have isolated a gene that co-segregated with the *Never-ripe* (Nr) locus<sup>31</sup>, which encodes a protein with homology to the *Arabidopsis* ethylene receptor ETR1 (Ref. 32) and prokaryotic two-component signal transducers (histidine kinases). The NR mRNA is positively regulated by ethylene during fruit development. In *Arabidopsis*, the two-component histidine kinase ETR1 is thought to function upstream from the Raf homolog CTR1 in the ethylene signaling pathway<sup>23,32</sup>. In higher plants, the mRNAs of various genes involved in signal transduction pathways, such as protein kinases, a phospholipase C, a histidine kinase, monomeric small GTP-binding proteins, calmodulins and transcription factors, accumulate in response to environmental stimuli or stresses<sup>33</sup>. Under environmental stress conditions, the elevated levels of the mRNAs of these putative signal transducers may increase their protein level, which is likely to amplify the signal transduction efficiency of the cascade. Analyses of transgenic plants that overexpress or repress genes for MAPKs, MAPKKs and MAPKKKs will help to elucidate the molecular mechanisms of the stress response and adaptation to environmental changes in higher plants.

#### References

- 1 Nishida, E. and Gotoh, Y. (1993) *Trends Biochem. Sci.* 18, 128-131
- 2 Cano, E. and Mahadevan, L. C. (1995) *Trends Biochem. Sci.* 20, 117-122
- 3 Herskowitz, I. (1995) *Cell* 80, 187-197
- 4 Jonak, C., Heberle-Bors, E. and Hirt, H. (1994) *Plant Mol. Biol.* 24, 407-416
- 5 Nishihama, R. *et al.* (1995) *Plant Cell Physiol.* 36, 749-757
- 6 Raingeaud, J., Whitmarsh, A. J., Barret, T., Dénjard, B. and Davis, R. J. (1996) *Mol. Cell. Biol.* 16, 1247-1255
- 7 Zhou, G., Bao, Z. Q. and Dixon, J. E. (1995) *J. Biol. Chem.* 270, 12665-12669
- 8 Millar, J. B. A., Buck, V. and Wilkinson, M. G. (1995) *Genes Dev.* 9, 2117-2130
- 9 Maeda, T., Takekawa, M. and Saito, H. (1995) *Science* 269, 554-558
- 10 Davenport, K. R., Sohaskey, M., Kamada, Y., Levin, D. E. and Gustin, M. C. (1995) *J. Biol. Chem.* 270, 30157-30161
- 11 Mizoguchi, T. *et al.* (1994) *Plant J.* 5, 111-122

- 12 Mizoguchi, T., Hayashida, N., Yamaguchi-Shinozaki, K., Kamada, H. and Shinozaki, K. (1993) *FEBS Lett.* 336, 440-444
- 13 Wilson, C., Eller, N., Gartner, A., Vicente, O. and Heberle-Bors, E. (1993) *Plant Mol. Biol.* 23, 543-551
- 14 Decroocq-Ferrant, V., Decroocq, S., Van Went, J., Schmidt, E. and Kreis, M. (1995) *Plant Mol. Biol.* 27, 339-350
- 15 Puert, B., Gawienowski, M., Ropp, T. and Jacobs, T. (1993) *Plant Cell* 5, 87-96
- 16 Jonak, C., Páy, A., Bögre, L., Hirt, H. and Heberle-Bors, E. (1993) *Plant J.* 3, 611-617
- 17 Statström, J. P., Altschuler, M. and Anderson, D. H. (1993) *Plant Mol. Biol.* 22, 83-90
- 18 Hirt, A. K. and Phillips, A. L. (1995) *Plant Mol. Biol.* 27, 1043-1052
- 19 Wilson, C., Anglmayer, R., Vicente, O. and Heberle-Bors, E. (1995) *Eur. J. Biol.* 233, 249-257
- 20 Jonak, C., Kiegl, S., Lloyd, C., Chan, J. and Hirt, H. (1995) *Mol. Gen. Genet.* 248, 686-694
- 21 Seo, S., Okamoto, M., Seto, H., Ishizuka, K., Sano, H. and Ohashi, Y. (1995) *Science* 270, 1988-1992
- 22 Shibata, W. *et al.* (1995) *Mol. Gen. Genet.* 246, 401-410
- 23 Kieber, J. J., Rothenberg, M., Roman, G. K. A., Feldmann, K. A. and Ecker, J. R. (1993) *Cell* 72, 427-441
- 24 Banno, H. *et al.* (1993) *Mol. Cell. Biol.* 13, 4745-4752
- 25 Mizoguchi, T. *et al.* (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 765-769
- 26 Knetoch, M. L. W., Wang, M., Snaar-Jagalska, B. E. and Heimovaara-Dijkstra, S. (1996) *Plant Cell* 8, 1061-1067
- 27 Suzuki, K. and Shinshi, H. (1995) *Plant Cell* 7, 639-647
- 28 Usami, S., Banno, H., Ito, Y., Nishihama, R. and Machida, Y. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 8660-8664
- 29 Bögre, L., Ligterink, W., Heberle-Bors, E. and Hirt, H. (1996) *Nature* 383, 489-490
- 30 Jonak, C. *et al.* (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 11274-11279
- 31 Wilkinson, J. Q., Lanahan, M. B., Yen, H.-C., Giovannoni, J. J. and Klee, H. J. (1995) *Science* 270, 1807-1809
- 32 Chang, C., Kwok, S. F., Bleecker, A. B. and Meyerowitz, E. M. (1993) *Science* 262, 539-544
- 33 Shinozaki, K. and Yamaguchi-Shinozaki, K. (1996) *Curr. Opin. Biotechnol.* 7, 161-167

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